Prevalence of Hepatitis G Virus in Patients with Hemophilia and Their Steady Female Sexual Partners

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Background: **Hepatitis G virus (HGV), also known as GB virus C, is a newly discovered Flavivirus that is transmissible by blood transfusion and other possible routes.**

Objective: **To study the risk of sexual transmission of HGV in female sexual partners of men with hemophilia (n = 161 couples).**

Methods: **Blood samples obtained from 11 medical centers were analyzed for (1) HGV RNA by polymerase chain reaction; (2) antibodies to HGV by enzyme immunoassay; and (3) other viruses and T-cell counts by routine laboratory tests. Subjects completed a questionnaire that assessed sexual intercourse frequency, number of sexual partners, condom usage, sexually transmitted diseases, illicit drug usage, and needlestick or broken-glass injuries.**

Results: **The HGV infection (RNA ± antibody positive) prevalence was 48% among men and 21% among women. Prevalence of hepatitis C virus, hepatitis B virus, and HIV among men was 99%, 94%, and 86%, compared with 3%, 11%, and 12% among women, respectively. The odds ratio for HGV infection for women with an HGV-positive male sexual partner was 2.14 ($P = 0.06$) without adjustment, and 2.77 ($P = 0.03$) with adjustment for other variables, none of which were independently significant.**

Conclusion: **These results suggest a low level of HGV sexual transmission.**

THE HEPATITIS G VIRUS (HGV)[1] and its strain variant, GB virus type C (GBV-C),[2] are members of the *Flaviviridae* family. These viruses are distantly related to the hepatitis C virus (HCV), with which they exhibit 29% amino acid-sequence homology. Although HGV and GBV-C have been associated with both acute and chronic hepatitis, their role in the causation of liver disease has been questioned,[3] and at present no clinically significant correlates have been clearly established. Regardless, the potential exists for intrahepatic or extrahepatic disease manifestations in a susceptible subset of patients, and may include an association with B cell non-Hodgkin's lymphoma. [4]

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The HGV/GBV-C agent is highly prevalent, with a carrier rate of 1% to 2% among healthy volunteer donors and 10% to 20% among populations at high risk for parenteral exposure.[1,3] The prevalence of antibody to the viral envelope has been twofold to fourfold higher than the prevalence of viremia; HGV can be transmitted via transfusion with prolonged viremia.[5] However, the relatively high prevalence suggests that nonparenteral modes of transmission may exist. This study addresses the possibility of sexual transmission using a cohort of highly exposed men with hemophilia and their female sexual partners. Sexual transmission of hepatitis B virus (HBV) (30% efficiency) and HIV (10–15% efficiency) have been well established,[6,7] whereas sexual transmission of HCV is rare.[8,9] Sexual transmission of HGV/GBV-C has been previously reported in one study of spouses[10] and in two studies of prostitutes.[11,12] Sexual transmission from men to women has also been suggested by the higher prevalence of HGV RNA or antibodies among female blood donors (5.2%) compared with male blood donors (3.3%).[13]

Previously collected blood samples from men with hemophilia and their long-term sexual partners and the availability of detailed sexual, drug-use, or other parenteral exposure histories allowed for an in-depth assessment of HGV-transmission patterns. In this assessment, we measured HGV RNA and an antibody to the HGV envelope protein, anti-E2.[14]

Methods

Subjects

Blood samples were drawn from 161 men with hemophilia and their wives or steady female sexual partners (n =

161). The data were collected from couples attending the Hershey Medical Center (n = 33), Cornell Medical Center (n = 10), Cardeza Medical Center (n = 24), Greece Hemophilia Medical Center in Athens (n = 2), Central Blood Bank of Pittsburgh (n = 9), Mount Sinai Medical Center (n = 14), Tulane Medical Center (n = 21), University of North Carolina, Chapel Hill Medical Center (n = 20), University Hospitals of Cleveland (n = 12), George Washington University Hospital (n = 15), and Austria Hemophilia Center in Vienna (n = 1). Institutional review board approval was obtained from the National Cancer Institute, the Research Triangle Institute (the coordinating data institute), and each of the participating medical centers. Signed informed consent was obtained from all participants.

Serum or plasma samples were obtained from each couple between 1986 to 1996 and stored at -70 °C. At the time of phlebotomy, most female participants had completed a self-administered questionnaire that assessed current sexual activity (i.e., during the previous 12 months) and other risk behaviors, such as the extent of injuries sustained while assisting with the partner's treatment, condom use, illicit drug use, and sexual promiscuity. Sexual promiscuity was defined as having had sexual relations with someone other than one's usual partner or by the presence of a sexually transmitted disease.

Detection of HGV RNA by Polymerase Chain Reaction and Anti-HGV E2 Antibody

Hepatitis G virus RNA was extracted from sera using an RNA isolator (Genosys Biotechnologies Inc., Woodland, TX) with 5 pg yeast tRNA and 40 μg glycogen to 100 μl serum. Extracted RNA was dissolved in 15 ml 12% dimethyl sulfoxide in distilled, deionized RNase-free water. In heparinized samples, 100 μl of the plasma sample was treated with heparinase,[15] followed by extraction of nucleic acids with an RNA isolator (Genosys Biotechnologies Inc.). The supernatant was precipitated with LiCl[16] and washed with 0.5 ml 80% ethanol twice using a silicon spin column (Qiagen Inc., Chatsworth, CA). The RNA was then eluted in 75 μl 12% dimethyl sulfoxide, and 15 μl RNA solution was used for polymerase chain reaction (PCR) assay.

The complimentary DNA from serum or plasma was synthesized from RNA with 1 U/μl Moloney murine leukemia virus reverse transcriptase in 20 μl 0.75 U/μl RNAase inhibitor, 50 mmol/l Tris-HCl, 40 mmol/l KCl, 6 mmol/l $MgCl_2$, 10 mmol/l dithiotheritol, 200 mmol/l each dATP, dCTP, dGTP and dTTP, and 10 μg hexanucleotide mix incubated at 42 °C for 30 minutes. The PCR method used was the PCR enzyme-linked immunoabsorbent assay DIG-labeling kit (Boehringer Mannheim GmbH, Indianapolis, IN). The 5′ noncoding region primers NCR-1 (5′-CTCTTTGTGGTAGTAGCCGAGAGA-3′) and NCR-2 (5′-CGAATGAGTCAGAGGACGGGGTAT-3′) were used for 60 cycles. This entailed incubation at 94 °C for 1 minute, 5 cycles at 94 °C for 45 seconds, 55 °C for 60 seconds, and 72 °C for 60 seconds, followed by 55 cycles 94 °C at 60 seconds, 55 °C for 60 seconds, and an additional 7 minutes at 72 °C for terminal extension.

The PCR products were detected by the Enzymn-Test DNA detection system (Boehringer Mannheim GmbH) on an automated ES 300 machine. Anti-HGV E2 antibody was detected by Enzymn-Test Anti-HGenv (Boehringer Mannheim GmbH), also on an automated ES 300 machine.

In this study, a person was classified as being currently or previously infected with HGV if he or she had detectable HGV RNA or anti-E2 antibody, respectively. Detecting viremia or anti-E2 antibody alone would fail to detect some infections, because these markers tend to be mutually exclusive. Viremia is cleared in more than half of acute infections, and is followed by the appearance of anti-E2 antibody that generally persists throughout long-term follow-up period.[12]

Other Laboratory Tests

Hepatitis B surface antigen, antibody to hepatitis B surface antigen and core antigen, anti-HCV antigens, and anti-HIV antigens were measured by commercial enzyme immunoassays according to the manufacturers' directions. Subjects with positive test results were classified as being infected with the corresponding virus. $CD4^+$ and $CD8^+$ lymphocyte counts were performed by flow cytometry using commercial reagents and standard techniques.

Statistical Techniques

Univariate analysis of potential risk factors for HGV infection in women was conducted with the chi-square test and using Yates correction or the Fisher exact test for small frequencies. For ordered nondichotomous factors, trends were assessed using the Mantel-Haenszel test. Finally, multivariate logistic regression was performed allowing for adjustment of potential confounders. All procedures were performed using SAS version 6.12 (SAS Institute, Cary, NC).

Results

Seventy-seven of the 161 hemophilic men (48%) had evidence of current or past HGV infection, including 22 men with only HGV RNA, 51 men with only HGV anti-E2 antibodies, and four men with both HGV RNA and anti-E2 antibodies. Thirty-four steady female sexual partners (21%) of the entire male cohort had evidence of HGV infection, including 17 women with only HGV RNA and 17 women with only HGV anti-E2 antibodies. In contrast, the prevalence of HCV, HBV, and HIV among men was 99%, 94%, and 86%, respectively (Table 1). Prevalence of past or

TABLE 1. Prevalence of Viral Infections

Infection	Hemophilic Men (n = 161)	Female Sexual Partners (n = 161)
Hepatitis G virus	48%	21%
Hepatitis C virus	99%	3%
Hepatitis B virus	94%	11%
HIV	86%	12%

current coinfection was 47% for HCV and HGV, 44% for HBV and HGV, and 40% for HIV and HGV. Among women, HCV, HBV, and HIV prevalence was 3%, 11%, and 12%, respectively (Table 1). Past or current coinfection rates among women were 1% for HCV and HGV, 4% for HBV and HCV, and 1% for HIV and HGV.

Twenty-one of the 34 HGV-positive women were sexually active with an HGV-positive hemophilic partner (seven men with HGV RNA and 14 men with only anti-E2 antibodies), whereas 13 HGV-positive women were not sexually active with an HGV-positive hemophilic partner. Of these 13 women, 7 women had a history of other sexual partners who were not tested for HGV, 2 women had a history of intravenous drug use or needle injury, and 4 women had no identified risk factor. Neither HGV RNA nor HIV infection in the male hemophilic partner was significantly associated with past or current HGV infection in the female partner (Table 2). Likewise, $CD4^+$ and $CD8^+$ lymphocyte counts and ratios in men and women were unrelated to HGV infection in the female partner.

Frequency of vaginal intercourse with an HGV-positive

TABLE 2. Associations Between Various Variables by Univariate Analysis

Variable	No. of Women*	Number HGV Infected (%)	P
Man's HGV RNA status			
Positive	26	7 (27%)	
Negative	135	27 (20%)	0.43
Man's HGV and HIV status			
Both positive	64	17 (27%)	
HGV positive, HIV negative	13	4 (31%)	
HGV negative, HIV positive	75	10 (13%)	
Both negative	9	3 (33%)	0.15
Man's HGV RNA and HIV status			
Both positive	22	6 (27%)	
Either or both negative	139	28 (20%)	0.45
Frequency of sex with an HGV-infected man			
Currently unexposed†	79	13 (16%)	
More than 3 per week	8	3 (37%)	0.05‡
1 per month or less	6	1 (17%)	
2–3 per month	16	4 (25%)	
4–5 per month	10	5 (50%)	
6–8 per month	12	2 (17%)	
2–3 per week	12	4 (33%)	
Condom use during sex with an HGV infected man			
Never use condoms (0%)	35	8 (23%)	
Occasionally use condoms (≤ 50% use)	18	6 (33%)	
Usually use condoms (> 50%)	30	7 (23%)	
Always use condoms (100%) or no sex	58	10 (17%)	0.37‡
Other sex partners			
None ever	61	10 (16%)	
< 10 episodes	73	16 (22%)	
≥ 10 episodes	12	4 (33%)	0.18‡
History of sexually transmitted disease			
None ever	146	34 (23%)	
At least once	7	0 (0%)	0.35
Parenteral exposure to drugs			
None ever	150	33 (22%)	
At least once	2	1 (50%)	0.40
Injury with glass/needle			
None ever	58	15 (26%)	
At least once	12	2 (17%)	0.72
Female CD4/CD8 lymphocyte ratio			
Low (< 1)	15	2 (13%)	
Normal (≥ 1)	104	21 (20%)	0.53

*$N \neq 161$ because of missing questionnaire data.
†Man is HGV-negative, or no intercourse in previous 12 months.
‡Trend test results.
HGV = hepatitis G virus.

TABLE 3. Logistic Model Relating Female HGV Status to Sexual Activity, Adjusting for Potential Confounders

Variable	No. of Women	Odds Ratio	95% CI
Sexual exposure			
Not currently exposed	79	1.00*	
Sex with with an HGV-infected man	64	2.77	1.11–6.96 ($P = 0.03$)†
Unknown sexual activity	18	0.14	0.007–2.80
Condom usage			
Always use condoms (100% use) or no sex	58	1.00*	
Usually use condoms (> 50% use)	30	1.06	0.33–3.42
Occasionally use condoms (≤ 50% use)	18	2.25	0.61–8.31
Never use condoms (0% use)	35	1.34	0.45–3.98
Unknown frequency of condom use	20	2.54	0.21–30.80
Parenteral exposure to blood or drugs			
None	58	1.00*	
Any	12	0.46	0.085–2.53
Unknown	91	0.80	0.34–1.90
Sexual activity with other men/STD			
None	61	1.00*	
Any	85	1.38	0.56–3.40
Unknown	15	4.79	0.86–26.89
Female CD4/CD8 lymphocyte ratio			
Normal ratio (≥ 1)	104	1.00*	
Abnormal ratio (< 1)	15	1.12	0.20–6.28
Unknown	42	1.69	0.65–4.42

The logistic model has a c value of 0.70. The Hosmer-Lemeshow goodness-of-fit statistic was 7.93 with 7 df (p = 0.34).
*Referent category.
†All other comparisons were not statistically significant.
HGV = hepatitis G virus; STD = sexually transmitted disease.

hemophilic man was significantly associated with HGV positivity in women ($P = 0.05$)(Table 2). Condom use and other postulated covariates appeared unrelated to HGV in women (Table 2). By univariate logistic regression, sexual intercourse with an HGV-positive man had an odds ratio of 2.14 (95% CI = 0.96–4.77, $P = 0.06$) for past or current HGV infection in women. By multivariate logistic regression model, the odds ratio was 2.77 (95% CI = 1.11–6.96, $P = 0.03$) with all postulated confounding variables included in the model (Table 3). None of these other variables were statistically significant in the multivariate model.

Discussion

Hepatitis G virus is a newly discovered member of the *Flaviviridae* family that is highly prevalent throughout the world, readily transmitted by transfusion and from mother to infant,[17] and can lead to persistent infection. Several studies have indicated that HGV infection does not appear to cause clinically significant hepatitis.[3] Extrahepatic manifestations of HGV are undefined, although it has been associated with a better prognosis for inpatients coinfected with both HIV and HGV.[18]

Because the plasma products that the hemophilic male participants received were not screened for HGV, it is surprising that the prevalence of HGV was only 48%, which is lower than the prevalence of HBV (94%), HCV (99%), and HIV (86%) in this population. One could speculate that HGV may be lost or inactivated during plasma fractionation, or that HGV is more effectively cleared by the host immune system than are other viruses.

Although parenteral and perinatal transmission of HGV seems unequivocal,[3,5,17] sexual transmission of this agent has not been established. We chose to study the potential for sexual transmission in a population in which males had a high exposure rate due to the need for life-long infusions of plasma clotting-factor concentrates, and in which the female sexual partners would be expected to have a low exposure rate. Among volunteer blood donors, the HGV prevalence was 3.3% in males and 5.2% in females.[13] In our study, the observed prevalence of past or current HGV exposure in the female partners of hemophiliacs was 21%, approximately fourfold to sixfold higher than in the general population. One could speculate that some women were infected with HGV from needle injuries while assisting with clotting factor infusions. However, this would appear to play a minor role, as judged by the women's questionnaire data and 3% prevalence for HCV, a virus that is readily transmitted by needle stick. Contrary to our expectations, HGV viremia (RNA) in men was not associated with an increased risk for their female partners ($P = 0.43$). However, our cross-sectional study design may have failed to detect a relationship between viremia in males and infection in females, especially when the average duration of the relationship was 9.7 years. A longitudinal design with serial testing of samples for the presence of HGV RNA and anti-E2

antibody might have revealed an association between HGV viremia and sexual transmission. The same study design limitations would contribute to the undetected yet possible benefit of condom use.

The performance of the assays used for detecting HGV RNA and HGV antibodies are not well defined, and are far from optimal. For example, although the manufacturer provided criteria for determining an absorbance cutoff value for HGV-antibody detection, this cutoff has not been rigorously and independently validated. We did note that the mean absorbance value for men who were antibody negative was higher than the mean absorbance value for their steady sexual partners. Hence, it is possible that some men may be antibody positive when using a different cutoff criterion. Additionally, the sensitivity of the HGV-RNA PCR assay differed by 1 log, depending on whether the sample was heparinized plasma (detection limit = 100 copies/ml) or serum (detection limit = 10 copies/ml); however, the male–female pairs were matched according to sample type. Such imperfections would underestimate the prevalence of HGV and weaken the detection of sexual or other associated variables, but they should not have introduced significant bias. No apparent bias was observed when the effect of HIV or HCV status on the detection of HGV RNA or anti-E2 antibody was analyzed (data not shown).

Previous studies conducted with prostitutes[11,12] and spouse pairs[10] have suggested that sexual transmission of HGV does occur. Our study of 161 couples is the largest study to date that included matched pairs and relevant questionnaire data. Our findings suggest that HGV infection rates in women are increased twofold to threefold if they have engaged in sexual intercourse with an HGV-infected man, which suggests that low-level sexual transmission of HGV does occur.

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